

**MULTI-PARTITE LIGANDS AND METHODS OF IDENTIFYING AND
USING SAME**

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

5 The present invention relates generally to medicinal chemistry and more specifically to agents which bind to more than one site on an enzyme.

 One of the major scientific undertakings of recent years has been the identification of genetic
10 information with the ultimate goal being the determination of the entire genome of an organism and its encoded genes, termed genomic studies. One of the most ambitious of these genomic projects has been the Human Genome Project, with the goal of sequencing the entire
15 human genome. Recent advances in sequencing technology have led to a rapid accumulation of genetic information, which is available in both public and private databases. These newly discovered genes as well as those genes soon to be discovered provide a rich resource of potential
20 targets for the development of new drugs.

 Two general approaches have traditionally been used for drug discovery, screening for lead compounds and structure-based drug design. Both approaches have advantages and disadvantages, with the most significant
25 disadvantage being the laborious and time-consuming nature of using these approaches to discovery of new drugs.

 Drug discovery and development based on screening for lead compounds involves generating a pool
30 of candidate compounds, often using combinatorial chemistry in which compounds are synthesized by combining

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a diagram representing bi-ligands bound to specific receptors. The bi-ligand contains three components, a common ligand, a specificity
5 ligand and an expansion linker. The common ligand, which binds to a conserved site in a receptor family, is designated by a pentagon. The specificity ligand binds to a specificity site on the receptor and is depicted as a triangle, square, circle and star for drugs 1 through
10 4, respectively. The expansion linker, indicated by two lines, bridges the common ligand and specificity ligand in an orientation allowing both the common ligand and specificity ligand to bind simultaneously to the
15 receptor.

Figure 2 shows a diagram of two different bi-ligands bound to two different receptors (top row), a bi-target ligand (middle row) and the same bi-target ligand bound to either target 1 or target 2 (bottom row).
20 The bi-target ligand contains four components, a common ligand, two specificity ligands, and an expansion linker. The common ligand, which binds to a conserved site in a receptor family, is designated by a pentagon. The specificity ligands of the bi-target ligand, designated
25 by a square and a triangle, bind to targets 1 and 2, respectively. The expansion linker, indicated by three lines, bridges the common ligand and the specificity ligands in an orientation allowing the common ligand and one of the specificity ligands to bind simultaneously to
30 its specific target. The bi-target ligand depicted can bind to the common site and specificity site on target 1 or the common site and specificity site on target 2.

phosphotransferases with a carboxyl group as an acceptor (EC 2.7.2); phosphotransfer with a nitrogenous group as an acceptor (EC 2.7.3); phosphotransferases with a phosphate group as an acceptor (EC 2.7.4); and
5 diphosphotransferases (EC 2.7.6).

Enzymes can also bind coenzymes or cofactors such as nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), thiamine pyrophosphate, flavin adenine dinucleotide (FAD)
10 and flavin mononucleotide (FMN), pyridoxal phosphate, coenzyme A, and tetrahydrofolate or other cofactors or substrates such as ATP, GTP and S-adenosyl methionine (SAM). In addition, enzymes that bind newly identified cofactors or enzymes can also be receptors.

15 As used herein, the term "receptor family" refers to a group of two or more receptors that share a common, recognizable amino acid motif. A motif can also be known as a pattern, signature or fingerprint. A motif in a related family of receptors occurs because certain
20 amino acid residues are required for the structure, function or activity of the receptor and are therefore conserved between members of the receptor family. The function or activity of a receptor can be enzymatic activity or ligand binding. Methods of identifying
25 related members of a receptor family are well known to those skilled in the art and include sequence alignment algorithms and identification of conserved patterns or motifs in a group of polypeptides, which are described in more detail below. Members of a receptor family also
30 bind a natural common ligand, which can be verified in a binding assay after the receptor is cloned and expressed.

above and in the public databases. Once a receptor family has been identified, a determination is made as to whether the receptor family is useful for identifying ligands as potential therapeutic agents. This is done by
5 determining if the receptor family has a natural common ligand that binds to at least two members of the receptor family, and preferably to several or most members of the receptor family.

In many cases, an identified receptor family
10 will have a natural common ligand that is already known. For example, it is known that dehydrogenases bind to dinucleotides such as NAD or NADP. Therefore, NAD or NADP are natural common ligands to a number of dehydrogenase family members. Similarly, kinases bind
15 ATP, which is therefore a natural common ligand to kinases. Other natural common ligands of a receptor family can be the coenzymes and cofactors described above.

After a receptor family has been determined, at
20 least two receptors in the receptor family are selected as drug targets for identifying ligands useful as therapeutic agents. The criteria for selection of receptor family members depends on the needs of the user. For example, if the receptor family is from a pathogenic
25 organism, the receptor family members selected can be those most divergent from the organism to be treated with the therapeutic agent. If the organism to be treated is a mammal such as human, then the receptor family members from the pathogenic organism are compared to known
30 mammalian or human members of the receptor family. Methods of comparing protein sequences are well known in the art and include BLAST as described above. Those receptors that are most distantly related to human can

10. The method of claim 9, further comprising:

(d) screening said population of bi-ligands for binding to a receptor in said receptor family; and

(e) identifying a bi-ligand that binds to and
5 has specificity for said receptor.

11. The method of claim 9, wherein said population comprises 3 or more bi-ligands.

12. The method of claim 9, wherein said population comprises 5 or more bi-ligands.

10 13. The method of claim 9, wherein said population comprises 10 or more bi-ligands.

14. The method of claim 9, wherein said population comprises 20 or more bi-ligands.

15 15. A method for generating a library of bi-ligands, comprising

(a) determining a common ligand to a combined specificity site-conserved site in a receptor family;

(b) attaching an expansion linker to said common ligand, wherein said expansion linker has
20 sufficient length and orientation to direct a second ligand to the specificity site of said combined specificity site-conserved site of a receptor in said receptor family, to form a module; and

(c) generating a population of bi-ligands comprising a plurality of identical modules attached to variable second ligands

wherein said bi-ligand exhibits at least 200-fold higher
5 affinity for one member of said receptor family over a second member of said receptor family.

16. The method of claim 15, further comprising:

(d) screening said population of bi-ligands
10 for binding to a receptor in said receptor family; and

(e) identifying a bi-ligand that binds to and has specificity for said receptor.

17. The method of claim 15, wherein said bi-ligand exhibits 300-fold higher affinity for one member
15 of said receptor family over a second member of said receptor family

18. The method of claim 15, wherein said bi-ligand exhibits 500-fold higher affinity for one member of said receptor family over a second member of said
20 receptor family

19. The method of claim 15, wherein said bi-ligand exhibits 1000-fold higher affinity for one member of said receptor family over a second member of said receptor family

25 20. The method of claim 15, wherein said combined specificity site-conserved site is selected from the group consisting of SH2 domain and SH3 domain.

mononucleotide, pyridoxal phosphate, coenzyme A, tetrahydrofolate adenosine triphosphate, guanosine triphosphate and S-adenosyl methionine.

65. The bi-target ligand of claim 62, wherein
5 said expansion linker has approximate C2 symmetry.

66. The bi-target ligand of claim 65, wherein
said expansion liner has perfect C2 symmetry.

